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(54) Title: IN OVO YEAST TREATMENT TO DIMINISH SALMONELLAE POPULATIONS IN POULTRY

(57) Abstract

To reduce the level of contamination of processed poultry, pathogen-free or nearly pathogen-free birds must be delivered to the processing plant. Therefore, it is important to prevent and reduce early contamination and spread of *Salmonella* in poultry. An in ovo method of treating embryos with a defined competitive exclusion preparation containing *Saccharomyces cerevisiae var. boulardii* is especially effective for reducing *Salmonella* colonization in newly hatched chicks.

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IN OVO YEAST TREATMENT TO DIMINISH SALMONELLAE POPULATIONS IN POULTRY

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to an *in ovo* method using yeast to diminish the frequency and extent of contamination by enteropathogenic foodborne pathogens in newly hatched birds.

Description of the Prior Art

Gastrointestinal pathogens figure prominently as the principal causes of human foodborne infections in many countries. Evidence supports the claim that poultry serves as an important reservoir for *Salmonella* in the food supply. As many as two million cases of salmonellosis occur annually in the United States (Stavric *et al*, Journal of Food Protection, Volume 56, No. 2, 173-180, February, 1993). The microorganisms may colonize poultry gastrointestinal tracts without any deleterious effects on the birds and, although some colonized birds can be detected, asymptomatic carriers can freely spread the microorganisms during production and processing, resulting in further contamination of both live birds and carcasses. Poultry serves as a primary reservoir for *Salmonella* and *Campylobacter* in the food supply (Jones *et al.*, Journal of Food Protection, Volume 54, No. 7, 502-507, July 1991, Jones *et al.*, Journal of Food Protection, Volume 54, No. 4, 259-262, April 1991). The intestinal contents of chickens may harbor up to 10^7 *Salmonella* and/or *Campylobacter* per gram, and cross contamination during processing is frequent (Oosterom *et al.*, Journal of Food Protection, Volume 46, No. 4, 339-344, April 1983). Studies have demonstrated that fecal material

constitutes the major source from which edible parts of chickens are contaminated in processing plants. Therefore, to significantly reduce the level of contamination on processed poultry, pathogen-free or nearly pathogen-free birds must be delivered to the processing plant (Bailey, Poultry Science, Volume 72, 1169-1173, 1993).

Better control measures are needed to minimize the spread of these and other human enteropathogenic bacteria; and the most promising approach to achieve this end has been to decrease the incidence and level of colonization by these microorganisms in poultry gastrointestinal tracts. To date, the most effective means for controlling *Salmonella* colonization is competitive exclusion (CE). Although the exact mechanism of CE protection is unclear, it is likely to be influenced by factors such as pH, Eh, production of inhibitory substances such as H_2S, bacteriocins, fatty acids, and conjugated bile acids; competition for nutrients and receptor sites; and local immunity (Mead *et al.*, Letters in Applied Microbiology, Volume 10, 221-227, 1990). Competitive exclusion treatment involves introduction of intestinal flora from pathogen-free adult birds into newly hatched chicks. A study by Nurmi *et al* (Nature, Volume 241, 210-211, January 19, 1973), first reported the use of the competitive exclusion technique. The reference discloses inoculation of 1 to 2 day old chicks by oral gavage with 1:10 dilution of normal intestinal flora and the birds were challenged with *Salmonella*. After 8-22 days, the birds were examined for the presence of *Salmonella*. It was found that only 33% of the treated birds were colonized with *Salmonella* whereas 100% of the untreated birds were colonized with *Salmonella*. Originally, a suspension of crop

and intestinal tract materials from healthy, adult birds was used. In later studies, cecal content was cultured anaerobically in a liquid medium. It was found that preparations of subcultured intestinal contents from healthy, adult birds conferred protection to young chicks whose intestinal or gut microflora had not yet been established. Administration of undefined CE preparations to chicks speeds up the maturation of the gut flora in newly-hatched birds and also provides a substitute for the natural process of transmission of microflora from the adult hen to its offspring.

There are many competitive exclusion treatments related to the use of undefined mixtures of organisms obtained from cecal contents or cecal wall scrapings which are subcultured (Snoeyenbos *et al.*, U.S. Patent No. 4,335,107; Mikkola *et al.*, U.S. Patent No. 4,657,762; Stern *et al.*, Avian Diseases, Volume 32, 330-334, 1988; Stern, Poultry Science, Volume 75, 402-407, 1994; and Stern *et al*, U.S. Patent Application Serial No. 08/031,983). While these undefined cultures have generally proven to be effective in reducing colonization of chickens with foodborne pathogens, there are concerns regarding their safety since there is the possibility of transmission of etiological agents associated with human foodborne disease and/or the transmission of avian disease.

U.S. Patent Application Serial No. 08/282,580 (Line *et al.*), herein incorporated by reference in its entirety, discloses a defined CE preparation of yeast which reduces the populations of gram-negative enteropathogenic *Campylobacter* and *Salmonella* in poultry by administering the CE preparation by oral gavage, in drinking water, in feed, by spraying newly hatched chicks with an aqueous suspension, or a combination of

the above. This treatment is most effective if administered as early as possible. There are numerous potential sources of salmonellae contamination in a modern poultry operation, including chicks, feed, rodents, birds, insects, and the transportation and processing procedures to which the birds are subjected. These sources of contamination make it difficult to administer a competitive exclusion culture to a bird before it is first colonized by microorganisms such as *Salmonella* and *Campylobacter*. Therefore, there is a need in the art to introduce a competitive exclusion culture as early as possible to poultry.

There have been a number of reports of *in ovo* vaccination of avian embryos. In U.S. Patent No. 5,206,015 (015), to Cox *et al.*, a method is disclosed for introducing an aqueous preparation of unattenuated probiotic bacteria into the digestive tract of a bird to exclude undesirable bacteria from colonizing the digestive tract. The bacterial culture is administered by depositing it in the air cell (large end) of an egg. The digestive tract of the hatchling of the inoculated eggs is found to be colonized by the bacterial culture at the time of hatch. In this method a hole is punched into the air cell end (large end) of the egg with a sterile needle, then the bacteria are administered using a smaller sterile needle, and finally the hole is either left unsealed or sealed with a bactieria-impermeable material.

U.S. Patent No. 4,458,630 ('630) to Sharma *et al.* discloses an embryonal vaccination for Marek's disease using unattentuated turkey herpesvirus (HVT) where the infection site is within either regions defined by the amnion or yolk sac. That is, the injection is midway along, and perpendicular to,

the longitudinal axis for amnion penetration through the large end of the egg with a one inch needle so that the needle passes through the outer and inner shell membranes enclosing the air cell and amnion and terminates in the fluid above the chick or in the chick itself. As in '015 patent, a hole is punched or drilled in the shell and this may be resealed with parafilm or the like.

U.S. Patent No. 4,040,388 ('388), to Miller teaches an automated method and apparatus for injecting embryonated eggs prior to incubation with a variety of substances into the albumin end (small end) of the egg. The reference teaches coagulative cooking of the surrounding albumin to seal the hole made by the injection. The drawbacks are that the vaccine is susceptible to inactivation during the heat coagulation step. Furthermore, Sharma *et al.* ('630) report that albumin has an inhibitory effect on the transport of an inoculant to the embryo at the egg's opposite end.

U.S. Patent No. 2,851,006 ('006) to Taylor *et al.*, teaches a method for increasing the hatch rate of bacterially infected eggs by means of *in ovo* treatment with a suitable bacteriophage in an aqueous preparation. The phage is introduced to the interior of the egg prior to incubation by any variety of techniques including by hyperdermic syringe, pressure differential in a dipping fluid and jet spray. With the hyperdermic syringe, a 26 gauge short shank needle is inserted at an oblique angle into the albumin end of the egg.

In U.S. Patent No. 3,120,834 ('834), Goldhaft *et al.* expands the application taught in Taylor to a variety of substances including antibiotics, sulfonamides, vitamins, enzymes, nutrients, and inorganic salts. These agents are

introduced through the shell prior to incubation by means of vacuum impregnation.

U.S. Patent No. 3,256,856 ('856) to Nicely *et al.* offers an improvement to the method of Goldhaft *et al.* in providing one or more holes in the egg shell for faciliting penetration. The hole(s) is (are) made in the air cell end (large end) of the egg, not extending beyond the inner shell membrane.

While there are various methods for *in ovo* introduction of microorganisms, there still remains a need in the art for the earliest protection of poultry to reduce the populations of gram-negative enteropathogenic *Campylobacter* and *Salmonella* in poultry. The present invention is different from prior art methods and provides for an *in ovo* method using a defined CE preparation of yeast.

SUMMARY OF THE INVENTION

It is therefore an object of the present invention to provide a method of *in ovo* treatment with a defined competitive exclusion composition to reduce pathogen colonization in poultry and, hence, on processed carcasses.

Another object of the present invention is to provide an *in ovo* treatment with a defined competitive exclusion composition which diminishes the presence of *Salmonella* in poultry and, hence, in processed carcasses.

A further object of the present invention is to provide an *in ovo* treatment with a defined competitive exclusion composition which includes *Saccharomyces* for diminishing the presence of *Salmonella* in poultry and, henc , in processed carcasses.

A still further object of the present invention is to provide poultry eggs which contain an effective amount of a defined competitive exclusion preparation to prevent or reduce colonization of newly hatched poultry by human enteropathogenic bacteria.

Another object of the present invention is to provide poultry eggs which contain *Saccharomyces* to prevent or reduce colonization of newly hatched poultry by *Salmonella*.

Further objects and advantages will become apparent from the following description.

DETAILED DESCRIPTION OF THE INVENTION

The importance of enteric infections in humans has been increasingly well recognized over the last dozen years. The relationship of poultry contamination and human infection has, likewise, become well documented. During broiler production and processing, fecal material containing pathogens are transferred onto meat and persist into food processing kitchens.

The application of yeasts as competitive exclusion microflora *in ovo* for the reduction of pathogen colonization has been discovered. *S. cerevisiae var. boulardii* is a non-pathogenic yeast originally isolated growing on lychee fruit in Indochina in the 1920's (Surawicz *et al.*, Gastroenterology, Volume 96, 981-988, 1989). Since 1962 it has been used in several countries to treat antibiotic-associated diarrhea in humans. It has been used widely in Europe and is under study in the U.S. for treatment of patients whose intestinal microflora has been compromised by intensive antibiotic therapy (Surawicz *et al.*, American Journal of Gastroenterology, Volume 84, 1285-1287, 1989; Gastroenterology, Volume 104, A786, 1993).

Often in these patients, antibiotic resistant pathogens take advantage of the lack of competing organisms and colonize the intestines of the patients causing severe and sometimes fatal diarrhea. Administration of the protective *S. cerevisiae var. boulardii* prevents toxin formation by gram-positive *Clostridium difficile* until the patients' normal protective microflora can be restored (Buts et al., Journal of Pediatric Gastroenterology and Nutrition, Volume 16, 419-425, 1993) and reduces the concentrations of several etiotogical agents of diarrhea (McFarland et al., Microbial Ecology in Health and Disease, Volume 6, 157-171, 1993).

There are several attributes of *S. cerevisiae var. boulardii* which indicate it has potential as a competitive inhibition composition in poultry. First, *S. cerevisiae var. boulardii* is rather thermophilic with an unusual optimum growth temperature of 37EC. It therefore is able to withstand the higher body temperature of poultry which is about 41.5EC for chickens. Second, the yeast has been shown to survive gastric acid in the stomach of mammals to reach the intestines (Bluehaut *et al.*, Biopharmaceutics and Drug Disposition, Volume 10, 353-364, 1989), which indicates that it might survive passage through the crop, proventriculus, and gizzard of chickens to reach the intestines and ceca. Third, it has demonstrated antagonistic activity *in vitro* and *in vivo* against various bacterial pathogens (Elmer *et al.*, Antimicrobial Agents and Chemotherapy, 129-131, January 1987); and last, *S. cerevisiae var. boulardii* can survive either aerobically or anaerobically, potentially making the culture and administration of the organism easier and more reliable than anaerobic cultures.

Experimental evidence now strengthens the theory that the binding of bacteria to host cell-surface sugars initiates infection (Sharon *et al.*, Scientific American, 82-89, January 1993). For example, *E. coli* have been demonstrated to exhibit a specific binding to the monosaccharide mannose. The host intestinal cells present mannose moieties which the *E. coli* attach to and initiate colonization of the intestines. *Salmonella* exhibit similar mannose-specific binding. The yeast, *S. cerevisiae var. boulardii*, contains high levels of mannose in its outer cell wall. It is believed that when the yeast is administered to chicks, the mannose presented by the yeast acts as a decoy to bind and agglutinate any *Salmonella* that may enter the gastrointestinal tract before the *Salmonella* can attach to the intestinal cell wall and initiate colonization of the bird. Since *S. boulardii* has been demonstrated not to permanently colonize poultry [unpublished data], the yeast and any yeast-bound *Salmonella* pass harmlessly out of the bird and *Salmonella* colonization is prevented.

The method of this invention is applicable to any avian animal whether domestic or wild and particularly to poultry that are raised for egg laying for human consumption which could serve as carrier for target pathogens. Poultry includes all domestic fowl raised for eggs or meat and includes chickens, turkeys, geese, ducks, pheasants, and the like.

The target pathogens include all human enteropathogenic bacteria capable of colonizing poultry. Of particular interest are *Salmonella*.

Yeast includes any species and strains of *Saccharomyces* such as *S. cerevisiae var boulardii*, other *S. cerevisiae*, *S. Carlsbergensis*, *S. ellipsoideus*, *S. intermedius*, for example,

and of particular interest is *Saccharomyces cerevisiae var. boulardii*.

Poultry eggs injected by the method of the present invention are fertile eggs which are preferably in the fourth quarter of incubation. Chicken eggs, for example, are treated on about the fifteenth to eighteenth day of incubation (the eighteenth day of embryonic development), and are most preferably treated on about the eighteenth day of incubation.

The site of injection of the defined yeast culture is preferably within either the region defined by the amnion or the air cell. Most preferably, the yeast culture is deposited in the air cell. The air cell is positioned at the large end of the egg adjacent the shell itself, and can be conveniently accessed by a shallow injection of approximately about 5 mm into the top of the large end of the egg. By the beginning of the fourth quarter of incubation, the amnion is sufficiently enlarged that penetration thereof is assured nearly all the time when the injection is made from the center of the large egg along the longitudinal axis.

The mechanism of injection is not critical, but it is preferred that the method not unduly damage the tissues and organs of the embryo or the extraembryonic membranes surrounding it so that treatment will not decrease hatch rate. A hypodermic syringe fitted with a needle of about No. 23 gauge is suitable. To inject into the air cell, the needle need only be inserted into the egg from just inside the inner surface of the egg to about seven millimiters under the shell. A pilot hole may be punched or drilled through the shell prior to insertion of the needle so that the inoculator will not have to push hard, running the risk that the final thrust will go too

deep or it will damage the shell and/or embryo. Furthermore, the pilot hole prevents damaging or dulling of the needle. If desired, the egg can be sealed with a substantially bacteria-impermeable sealing material such as wax or the like to prevent subsequent entry of undesirable bacteria.

An automated high speed injection system is suitable for implementing the present invention. Numerous devices are available such as those disclosed in U.S. Patent Nos. 4,040,388, 4,469,047, 4,593,646, and 5,176,101 as well as European Patent Application No. 87305746.7.

An effective amount of yeast in an aqueous solution is defined as the amount of yeast required to prevent colonization of enteropathogenic microorganisms in newly hatched chicks. A preferred dose range of yeast is approximately about 10^8 to 10^{10} yeast per egg per 0.1 ml with a more preferred dose of 10^9 yeast per egg per 0.1 ml.

The following examples are intended only to further illustrate the invention and are not intended to limit the scope of the invention as defined by the claims.

EXAMPLE 1

Preparation of the Yeast Competitive Exclusion Culture

Yeast are prepared by growing and harvesting pure cultures of *Saccharomyces cerevisiae var. boulardii* using conventional microbiological culture, fermentation, and cell collection techniques well known to those of skill in the art.

Saccharomyces cervisiae var. boulardii (s.b.) (formerly *S. boulardii*) [formerly ATCC #74012] has been redeposited as ATCC 74352 (American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852-1776. It is propagated in liquid

culture using Sabouraud dextrose broth (SDB, Difco, Detroit, MI) or any other suitable liquid mycological culture media. The yeast may also be grown on an agar surface using Sabouraud dextrose agar (SDA, Difco, Detroit, MI) or any other suitable mycological agar. Yeast grown in liquid culture medium is harvested by centrifugation and diluted in buffered peptone (BP) solution for delivery to the eggs. Yeast grown on agar surfaces may be harvested with a sterile cotton-tipped swab and diluted in BP solution for delivery to the eggs.

Alternatively, another strain *S. cervisiae var. boulardii* obtained from Lallemand, Inc. has been deposited at the American Type Culture Collection (12301 Parklawn Drive, Rockville, Maryland 20852-1776) as ATCC 74351. This strain may also be purchased from Lallemand, Inc. (1620 Préfontaine, Montréal, Québec, Canada HIW2N8) in a liquid form. The yeast is grown in a fermenter under simulated commercial conditions using sterilized molasses as the primary carbohydrate source. The yeast population in liquid form is enumerated and found to contain 10^{10} cfu/ml. It is stored at 40EC until use.

EXAMPLE 2

Test for Yeast Efficacy Against Salmonella Colonization

The efficacy of yeast treatments in preventing colonization of broiler chicks by *Salmonella* was tested. In this experiment, 150 fertile eggs were procured from a local broiler hatchery on the 18th day of incubation and randomly divided into 5 groups of 30 eggs each. Using an electric drill with a small (1 mm diameter) abrasive bit, a single hole was drilled in the large end of the eggs. Two groups of eggs served as yeast-treated groups and received 0.1 ml/egg of an *S.*

cerevisiae var. boulardii containing about 10^{10} viable yeast/ml. The yeast were delivered into the air cell of the egg using a 1cc syringe and a 23 guage needle to penetrate just below the surface of the shell (-5mm). Another 2 groups of eggs also served as yeast-treated groups and received 0.1ml/egg of a solution containing a lesser amount of *S. cerevisiae var. boulardii* (about 10^8 viable yeast/ml). A fifth group of eggs served as controls and received 0.1ml/egg of a buffered peptone (BP) solution (Juven *et al.*, 1984) in a similar manner. The eggs were transferred into small (approximately 80 cm^2) table-top egg incubators equipped with heaters, thermostats and circulating fans. The incubators were maintained at 99EC until the eggs hatched on day 21. When the majority of the eggs were hatched the tops of the incubators were removed and 10ml of a BP solution containing $6.7x10^3$ *Salmonella typhimurium* (resistant to 200 ppm nalidixic acid) were sprayed on the chicks and interior of each incubator. The tops of the incubators were replaced and the chicks remained in the hatching environment and were exposed to the *Salmonella* during this time. After 12 h the chicks were removed from the incubators. Like-treated chicks were grouped together and transferred into 3 separate isolation units equipped with nipple drinkers and a filtered air supply. The chicks were given a standard broiler starter feed and water *ad libitum*. After 36 h and again on day 8 post hatch, half of the chicks were killed by cervical disarticulation. The ceca were aseptically removed from each chick and placed in small individual stomacher bags. The ceca and contents were diluted 1:3 in sterile BP and blended for 30 seconds in a Waring blender. A 10^{-1} and 10^{-3} dilution of the ceca and contents was swabbed on brilliant green sulfa (BGS)

agar containing 200 ppm nalidixic acid for recovery of *Salmonella*. The agar plates were incubated at 35EC for 24h prior to enumerating *Salmonella* colonies. The bags containing the ceca were incubated for 24h at 35EC for enrichment and recovery of low populations of *Salmonella*. Samples from these bags were swabbed directly on BGS plates to detect colonies which might not have been detected by the direct plating method.

The results from this trial are shown below in Table 1. A 100% reduction in the incidence of *Salmonella* colonization in chicks hatched from yeast-treated eggs was observed upon analysis on day 7. All control chicks were colonized with *Salmonella*; whereas, none of the *in ovo* yeast-treated chicks were colonized. A reduction in *Salmonella* levels of 4.7 log were noted in the chicks receiving *S. cerevisiae var. boulardii in ovo* as compared to chicks receiving no yeast treatment. Chicks hatched from eggs receiving 10^9 viable yeast/egg were more resistant to *Salmonella* colonization than chicks hatched from eggs receiving 10^7 viable yeast/egg. Hatchability was not affected as 87% of the eggs hatched in both the 10^9 treated and control group.

TABLE 1

Diminished *Salmonella* colonization of chickens due to *in ovo* yeast treatment

Group	*In Ovo* Treatment	# of Eggs	Hatchability (%)	36h Analysis		Day 7 Analysis	
				% Birds Sal Pos	Overall Log Mean	% Birds Sal Pos	Overall Log Mean
1	10^7S.b.	59	73	85	2.63	100	3.55
2	10^9S.b.	60	87	12	+1.0	0	0
3	BP Control	60	87	100	4.22	100	4.73

EXAMPLE 3

S. cervisiae var. boulardii was kept at 4EC for 11 days and then administered as in example 2. 1.7×10^3 cfu *Salmonella* was sprayed into each incubator onto chicks and interior on day of hatch as in example 2. Thirty-six hours and day 8 post hatch, the chicks were killed by cervical disarticulation and the ceca aseptically removed. The tissue was analyzed for *Salmonella* presence as described above in example 2. The results are shown below in Table 2.

TABLE 2

Diminished *Salmonella* colonization of chickens due to *in ovo* yeast treatment

Group	*In Ovo* Treatment	# of Eggs	Hatchability (%)	36h Analysis		Day 7 Analysis	
				% Birds Sal Pos	Overall Log Mean	% Birds Sal Pos	Overall Log Mean
1	10^7S.b.	60	83	55	0.88	47	1.64
2	10^9S.b.	60	85	80	1.0	37	0.91
3	BP Control	30	87	58	1.16	92	4.29

In Table 2, there is a reduction in the incidence of *Salmonella* colonization in chicks 7 days post-hatch, hatched from eggs treated with a yeast preparation kept at 4EC for 11 days.

The foregoing detailed description is for the purpose of illustration. Such detail is solely for that purpose and those skilled in the art can make variations therein without departing from the spirit and scope of the invention.

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description
on page 11 and 12, line 27-28 and 12-14.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☐

Name of depositary institution
American Type Culture Collection

Address of depositary institution *(including postal code and country)*
12301 Parklawn Drive
Rockville, Maryland 20852-1776
US

Date of deposit	Accession Number
September 27, 1995	ATCC 74351 and ATCC 74352

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet ☐

2 microorganisms are disclosed in the specification and listed above. In respect to those designations in which a European Patent is sought, a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC).

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

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CLAIMS

What is claimed is:

1. A method for treating poultry *in ovo* to protect against colonization by human enteropathogenic bacteria in newly hatched poultry comprising

puncturing an egg of a bird containing a viable embryo with a puncturing means in a region of the air cell of said egg in order to administer a viable defined competitive exclusion preparation comprising *Saccharomyces*,

injecting said preparation into the egg by inserting a needle into said air cell, and

hatching said egg to obtain pathogen-free or nearly pathogen-free poultry.

2. The method of claim 1 wherein said egg is selected from the group consisting of chicken eggs, turkey eggs, geese eggs, duck eggs and pheasant eggs.

3. The method of claim 1 wherein said defined preparation contains an effective amount of *Saccharomyces* to prevent colonization of human enteropathogenic bacteria in newly hatched poultry.

4. The method of claim 3 wherein said *Saccharomyces* is selected from the group consisting of *S. cerevisiae var. boulardii*, *S. cerevisiae*, and mixtures thereof.

5. The method of claim 1 wherein said human enteropathogenic bacteria comprises *Salmonella*.

6. A poultry egg produced by the method of claim 1.

7. A poultry egg produced by the method of claim 3.

8. A poultry egg produced by the method of claim 4.

9. A poultry egg produced by the method of claim 5.

10. A poultry egg comprising an effective amount of a defined competitive exclusion preparation containing *Saccharomyces* to prevent colonization of newly hatched poultry by human enteropathogenic bacteria.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/16335

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A01K 33/00; A01N 63/00; A23L 1/32;
US CL :424/93.51; 119/6.8; 426/298;
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/93.51, 93.2, 93.1, 93.21; 119/6.8, 348; 426/298, 614; 435/172.3; 800/2, DIG.2, DIG.4, DIG.5, DIG.6;

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	LINE et al. Saccharomyces treatment to diminish Campylobacter and Salmonella in chickens. Atlanta, Georgia: Poultry Science. 1995, Vol. 74 (Suppl 1), page 201, S81. See entire entry.	1-10
Y	LINE et al. Treatment of feed with Saccharomyces boulardii for reduction of Salmonella populations in broiler chickens. Alberta, Canada: Poultry Science. 1995, Vol. 74 (Suppl. 1), page 49 146. See entire entry.	1-10

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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Date of the actual completion of the international search	Date of mailing of the international search report
20 DECEMBER 1996	21 JAN 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer DEBORAH CLARK Telephone No. (703) 308-0196

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/16335

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	COX et al. Research note: in ovo administration of a competitive exclusion culture treatment to broiler embryos. Poultry Science. October 1992, Vol. 71, No. 10, pages 1781-1784. See entire document, especially the abstract.	1-10
Y	US 5,438,954 A (PHELPS ET AL) 08 August 1995 (08/08/95), see entire document, especially Col. 1 lines 26-28.	1-10
Y	US 5,206,015 A (COX ET AL) 27 April 1993 (27/04/93), see entire document, especially the abstract.	1-10

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS, MEDLINE, CAPLUS, BIOSIS, CABA, WPIDS, EMBASE, FSTA
search terms: poultry, avian, bird, chicken, in ovo, saccharom?, yeast, s. cerevisi?, bacteria, salmonella, compet?, passive immunity, and inventors names